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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/501,777	07/19/2004	John Robert Birch	BJS-4145-14	5040

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EXAMINER	
EPPS FORD, JANET L	

ART UNIT	PAPER NUMBER
1633	

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01/15/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)
	10/501,777	BIRCH ET AL.
	Examiner	**Art Unit**
	Janet L. Epps-Smith	1633

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on 16 July 2008.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1,3-7,10,11,14,15,17-24 and 27-29 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1,3-7,10,11,14,15,17-24 and 27-29 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date 11/25/08;10/06/08;5/07/08.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

1. Claims 1, 3-7, 10, 14-15, 17-24, 27-29, 32-35 are under examination.

2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Response to Arguments

Claim Rejections - 35 USC § 103

3. Applicant's arguments with respect to the rejection of claims 1, 3-7, 10-11, 19-24 and 27 under 35 U.S.C. 103(a) as being unpatentable over Bebbington et al (U.S. Patent No. 5,891,693, of record) as evidenced by Barsomian et al (U.S. Patent No, 5,238,821, of record) in view of Brandt et al (U.S. Patent No. 6,395,484, of record), are moot in view of the new grounds of rejection set forth below.

Claim Rejections - 35 USC § 103

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. Claims 1, 3-7, 10, 14-15, 17-24, 27-29, and 32-35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wilson et al. (WO 87/04462 A1) or Bebbington et al (U.S. Patent No. 5,891,693, of record) as evidenced by Barsomian et al (U.S. Patent No, 5,238,821, of record) in view of Brandt et al (U.S. Patent No. 6,395,484, of record) and Gawlitzek et al. (see IDS filed 5/07/08) and Hermitin et al., are moot in view of the new grounds of rejection set forth below.

Wilson et al. teach the use of a recombinant DNA sequence, using glutamine synthetase as a selectable marker in glutamine auxotrophic cells to express tissue plasminogen activator (tPA; see page 8, lines 4-33; pages 9-11; Figure 3) Barsomian et al disclose that tPA is a sialylated glycoprotein (see column 2, lines 49-52, for example).

As stated in the prior Office Actions:

Bebbington et al teach mouse and rat lymphoid cell lines that can be transformed to glutamine independenceby incorporating a gene encoding glutamine synthetase (GS) so that the cells can grow in glutamine-free medium. Bebbington et al teach that the cell preferably contains a gene coding for a heterologous protein in which the gene is encoded on a separate vector (see column 2, lines 8-20, 25-30 and 44-51, for example), which meets the limitation of exogenous DNA sequences being located on more.than one DNA construct. Bebbington et al teach examples of heterologous proteins as human growth hormone, or tPA or tissue inhibitor of metalloproteinase (see column 2, lines 31-36, for example). Bebbington et al teach that the expression of the heterologous protein is substantially increased by selection for GS gene amplification (see column 2, lines 52-55, for example), which meets the limitation of GS as a selection marker. As stated above, Barsomian et al disclose that tPA is a sialylated glycoprotein (see column 2, lines 49-52, for example).

Bebbington et al do not teach a human glutamine-auxotrophic cell. Bebbington et al do not teach an exogenous sequence encoding a sialylated protein that further comprises a selectable marker such as claimed. Bebbington et al do not teach growing the cells in serum-free medium.

Brandt et al teach human cells (HT1080) for the preparation of human proteins in order to produce human proteins in economical yields in a form that is suitable for production of a pharmaceutical preparation (see column 1, lines 12--17, for example) which meets the limitation of glutamine-auxotropic human cell line or an immortalized fibrosarcoma HT1080 cell line. Brandt et al teach the advantage of using serum free culture medium for culture of human cells because purification of proteins from serum free culture is substantially easier and has no danger of contamination with animal pathogens (see column 2, lines 22-34, for example) which meets the limitation of a human cell growing in a serum free culture. Brandt et al teach that it is advantageous to use a human cell.line that synthesizes a desired protein with a glycosylation protein, especially a sialic acid protein comparable to that of the naturally occurring target protein (see column 3, lines (19030, for example).

Brandt et al also teach that a negative or positive selection marker or amplification gene can be included and can be DHFR, adenosine deaminase, ornithine decarboxylase or a thymidine kinase gene (see column 4, lines 56-65, for example)," which meets the limitation of a gene further comprising a selectable marker selected from the group

consisting of DHFR, adenosine deaminase, asparagine synthetase, aspartate transcarbamylase, metallotionein-1, ornithine decarboxylase, P-glycoprotein, ribonucleotide reductase, thymidine kinase and xanthine-guanine phosporibosyl transferase.

It would have been obvious to the skilled artisan at the time the invention was made to modify the teaching of Bebbington et al from making and using a rodent glutamine auxotrophic cell to produce an exogenous sialylated protein such as tPA to a human cell as taught by Brandt et al to produce a human exogenous sialylated protein because Brandt et al disclose that human cells are preferred for the preparation of human proteins in order to produce human proteins in economical yields in a form that is suitable for production of a pharmaceutical preparation. The motivation to use a human glutamine auxotrophic cell that is transfected with a glutamine synthetase sequence and a separate vector comprising a sequence for an exogenous sialylated protein is the expected benefit of being able to synthesize a target protein with a sialic acid moiety glycosylation pattern that is comparable to that of the naturally occurring target protein (see Column 3, lines 23-30, for example).

It also would have been obvious to the skilled artisan at the time the invention was made to modify the teaching of Bebbington et al from making and using a glutamine auxotrophic cell that is capable of growing in serum-free medium because Brandt teach the importance of serum-free medium for cultivation. The motivation to use a serum-free medium is the expected benefit of being able to reduce the danger of contamination of the protein produced with animal pathogens that might be introduced by, using animal serum (see column 2, lines 22-35, for example).

It also would have been obvious to the skilled artisan at the time the invention was made to modify the teaching of Bebbington et al and use a second exogenous DNA sequence as a selection marker and amplification gene because Brandt et al teaches clone selection and gene amplification using a positive or negative selection marker. The motivation to use a selection marker and amplification gene such as DHFR is the expected benefit as disclosed by Brandt et al of being able to use a gene with a sensitivity for a selection agent in order to increase the expression of an gene to be produced by culturing the cell in the presence of increasing concentrations of a selection agent (i.e. methotrexate) (see column 10, lines 36-44, for example). There is a reasonable expectation of success to make and use a human glutamine auxotrophic cell wherein these exogenous DNA sequences are located on more than one DNA vector construct, because it has worked previously in the references cited. Therefore Bebbington et al as evidenced by Barsomian et al in view of Brandt et al render obvious a human cell comprising an exogenous sequence encoding a sialylated protein (e.g. tPA) further comprising a selectable marker (e.g. DHFR) and an exogenous glutamine synthetase sequence wherein these sequences are on more than one DNA vector and the cell is capable of producing the protein and capable of growing in a glutamine-free and serum-free medium (claim 1).

Brandt et al teach an HT1080 cell line (see column 8, lines 24-50, for example), which meets the limitation of a glutamine-auxotropic human cell line or an immortalized fibrosarcoma HT1080 cell line (claims 3-5).

Brandt et al teach that the cells are cultured in serum-free medium and in suspension (see column 6, lines 25-42, for example). Bebbington et al teach that cells can be transformed to glutamine independence by incorporating a gene encoding GS so that the cells can grow in glutamine-free medium. Therefore, the cells made obvious by Bebbington et al in view of Brandt et al would be capable of growing in suspension in serum-free and glutamine-free medium (claim 6).

Brandt et al teach that the cells were. cultured to produce the target protein, which was then recovered and quantified from the cell supernatant (see column 6, lines 44-63, for example), which meets the limitation of a process for the production of a sialylated protein in a glutamine-auxotropic human cell in a serum free medium and recovery of the protein (claim 7).

As discussed above, Bebbington et al in view of Brandt et al render obvious a cell capable of growing in serum free and glutamine free medium and would also render obvious a process for producing a sialylated protein by culturing a glutamine-auxotrophic cells wherein the culture medium is serum-free and glutamine free (claims 10-11). Brandt et al teach production of a protein such as EPO using an HT1080 cell line (see column 8, lines 24-50, for example), which meets the limitation of a process wherein the cells is a glutamine-auxotropic human cell line or an immortalized fibrosarcoma HT1080 cell line (claims 19-20 and 25-27).

None of the cited references teach a method for increasing sialylation and/or N-glycan charge of a glycosylated protein. Furthermore, none of the cited references teach that sialylation is defined by N-glycan charge or that the sialylated protein comprises tri-, tetra- or pentasialo glycoforms of N-glycan.

Gawlitzek et al. teach that in the culture of mammalian cells, the metabolite ammonium is produced as a by-product of glutamine metabolism and the thermal degradation of glutamine. Gawlitzek et al. further teaches that increased amounts of ammonium in cells leads to a decrease in terminal galactosylation and sialylation of

TNFR-IgG. Thus, the reference provides a suggestion for increasing sialylation and/or N-glycan charge of a glycosylated protein in a cell without adding glutamine. Moreover, Gawlitzek et al. teach that increasing the N-glycan of a glycosylated protein, i.e. increasing the ratio of glycosylation of a glycosylated protein, decides the activity of the protein.

Hermentin et al teach a process for characterizing the glycosylation of glycoproteins based on a hypothetical charge number N. Hermentin et al teach that it is important to reliably determine the degree of glycosylation or sialylation in glycoproteins, such as erythropoietin, in order to gauge bioavailability/biological activity of a protein for therapeutic use. Hermentin et al discloses that when erythropoietin is incompletely glycosylated, it is quickly cleared from the blood circulation and would not be biologically useful (see column 1, lines 6-15, 28-45 and column 2, lines 14-25, for example). Hermentin et al teach that it is crucial to determine the distribution of glycan groups exhibiting differing degrees of sialylation to be able to index the bioavailability of a glycoprotein. Hermentin et al teach that the N charge of a glycoprotein is determined in part by determining the percentage of trisialo, tetrasialo and pentasialo ranges (see column 3, lines 27-50, column 4, lines 27-35, column 5, lines 4-12, for example). Hermentin et al teach that the N-glycan charge value was determined for EPO and erythropoietin is comprised of trisialylated N-glycans and tetrasialylated glycans (see column 12, lines 43-52, for example), which meets the limitation of EPO as a sialylated protein comprising tri and tetrasialylated glycoforms defined by N-glycan charge and a process of defining sialylation by N-glycan charge.

It would have been obvious to the skilled artisan at the time the invention was made to combine the cited references in the design of the instant invention. One of ordinary skill in the art would have been motivated to modify the method taught by Bebbington et al from making a rodent glutamine auxotrophic cell to produce an exogenous sialylated protein such as tPA to a human cell as taught by Brandt et al to produce a human exogenous sialylated protein because Brandt et al disclose that human cells are preferred for the preparation of human proteins in order to produce human proteins in economical yields in a form that is suitable for production of a pharmaceutical preparation. The motivation to make a human glutamine auxotrophic cell that is transfected with a glutamine synthetase sequence and a separate vector comprising a sequence for an exogenous sialylated protein is the expected benefit of being able to use the cells to synthesize a target protein with a sialic acid moiety glycosylation pattern that is comparable to that of the naturally occurring target protein (see column 3, lines 23-30, for example). Moreover, Wilson et al. expressly teach the exogenous expression of glutamine synthetase in glutamine auxotrophic cells.

It also would have been obvious to the skilled artisan at the time the invention was made to modify the method and cell taught by Bebbington et al and incorporate a second exogenous DNA sequence as a selection marker and amplification gene because Brandt et al teaches clone selection and gene amplification using a positive or negative selection marker. The motivation to use a selection marker and amplification gene such as DHFR to make a cell is the expected benefit as disclosed by Brandt et al of being able to use a gene with a sensitivity for a selection agent in order to increase

the expression of an gene to be produced by culturing the cell in the presence of increasing concentrations of a selection agent (i.e. methotrexate) (see column 10, lines 36-44, for example). There is a reasonable expectation of success to make a human glutamine auxotrophic cell wherein these exogenous DNA sequences are located on more than one DNA vector construct, because similar methods have worked previously in the references cited. Claim 28 appears to recite limitations regarding the order of the vector transformations. Limitations of this type would amount to routine optimization of the method. Therefore, Bebbington et al in view of Brandt et al render obvious a method to produce the glutamine-auxotropic human cell of claim 1 as claimed in claim 28.

It also would have been obvious to the skilled artisan at the time the invention was made to modify the method taught by Bebbington et al and make a glutamine auxotrophic cell that is capable of growing in serum-free medium because Brandt teach the importance of serum-free medium for cultivation. The motivation to use a serum-free medium is the expected benefit of being able to reduce the danger of contamination of the protein produced with animal pathogens that might be introduced by using animal serum (see column 2, lines 22-35, for example). There is a reasonable expectation of success to make a human glutamine auxotrophic cell with these limitations, because similar method have worked previously in the references cited. Therefore Bebbington et al as evidenced by Barsomian et al in view of Brandt et al render obvious a method to produce the glutamine-auxotropic human cell of claim 1 wherein the cell is further adapted to growth in serum free medium as claimed in claim 29.

It would have been obvious to the skilled artisan at the time the invention was made to determine the N-glycan charge for the sialylated protein such as EPO or tPA being produced by the cell because Hermentin et al teach that it is important to know the degree of glycosylation of recombinant therapeutic proteins such as erythropoietin, since slightly altered glycosylation patterns can drastically effect the activity of the therapeutic protein. The motivation to determine the N-glycan charge is the expected benefit of being able to determine the degree of glycosylation in a simple, reliable manner suitable for replacing the methods previously known in the art for determining the bioavailability of a therapeutic protein before use. There is reasonable expectation of success in combining the protein production method using a glutamine auxotrophic cell rendered obvious by Bebbington et al in view of Brandt et al with the methods taught by Hermentin et al and use a glutamine- auxotropic human cell transfected with an exogenous DNA sequence encoding a glycoprotein, such as erythropoietin, to produce and recover erythropoietin and determine its bioavailability via N-glycan charge, because these methods have worked before in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore, Bebbington et al as evidenced by Barsomian in view of Brandt et al and further in view of Hermentin et al render obvious a cell and a process for the production of a sialylated protein comprising culturing a glutamine-auxotropic human cell in glutamine free medium and recovering the expressed sialylated protein comprising tri-,

or tetrasialo glycoforms of the glycan defined by an N-glycan charge (claims 14-15 and 17-18).

Additionally, it would have been obvious to the ordinary skilled artisan at the time of the instant invention, to modify the teachings of Wilson et al. (WO 87/04462 A1) or Bebbington et al (U.S. Patent No. 5,891,693, of record) as evidenced by Barsomian et al (U.S. Patent No, 5,238,821, of record) in view of Brandt et al (U.S. Patent No. 6,395,484, of record) by the teachings of Gawliztek et al. The ordinary skilled artisan seeking to increase the sialylation and/or N-glycan charge of a glycosylated protein, would have been motivated to express the desired protein in a cell without adding glutamine, since the prior art clearly teaches that ammonium, a byproduct of glutamine metabolism has a direct effect on the level of sialylation and/or N-glyclan charge of a glycosylated protein.

Conclusion

6. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

7. Applicant's submission of an information disclosure statement under 37 CFR 1.97(c) with the fee set forth in 37 CFR 1.17(p) on 5/07/08 prompted the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE**

FINAL. See MPEP § 609.04(b). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

8. A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Janet L. Epps-Smith whose telephone number is 571-272-0757. The examiner can normally be reached on M-F, 10:00 AM through 6:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Janet L. Epps-Smith/
Primary Examiner, Art Unit 1633